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An Improved Method of Using Plant Pigment as an Indicator of Digestibility 1/

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The plant pigment method of determining digestibility is based on a mixture of pigments which appear to vary from plant to plant. Greater precision would be obtained in digestibility studies by the plant pigment method if a single plant pigment were used, instead of a heterogeneous collection. A single pigment, such as pheophytin, would have the same light transmission characteristics whether isolated from the forage or resulting feces. This pigment peaks at one point so that by reading at the peak wavelength, errors due to the presence of other pigments, and errors due to reading at a point where the slope of the absorption curve is steep, are diminished.

Irvin et al. have shown that the most significant plant pigment in cow feces (animals on all-alfalfa ration) was pheophytin. Pheophytin is the first decomposition product of chlorophyll, being formed by the removal of magnesium from the chlorophyll molecule. If the absorption of chlorophyll by the animal is low, less than 5%, and if the chemical change undergone by the chlorophyll in the bovine intestinal tract can be duplicated in the laboratory, (chlorophyll changed to pheophytin) then it would be possible to determine the pheophytin in the forage fed and in the resulting feces, and from these data calculate the digestibility of the forage by ratio technique.

After some experimentation it was found that a treatment of the plant pigments with oxalic acid would change the chlorophyll of the forage to pheophytin.

The plant pigments were extracted from the feed and feces in the usual manner with 85% acetone or 95% alcohol. The solutions containing the extracted pigments were made to proper volume. Aliquots of the orchard grass and clover extracts were diluted with a 4% NaCl solution and extracted with Skelly F. which was evaporated and taken up in alcohol. These alcohol solutions were treated with a few ml. of saturated oxalic acid and filtered. For pasture grasses, the addition of oxalic acid caused a color change from green to yellow, while the feces solutions, already yellow, did not change. Solutions were then read in a spectrophotometer at 415 millimicrons, the wavelength at which pheophytin displays its maximum absorption point.

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It is assumed that although other pigments may be present, pheophytin is the predominant pigment. Figure 1 demonstrates the effect of the addition of a chelating agent on the absorption curve of forage pigments, and the natural peaking of the feces pigments at 415 millimicrons. To test the feasibility of using pheophytin as an indicator of digestibility, the following experiments were conducted.

Three cows were placed in digestion stalls and fed a ration of orchard grass clippings. These clippings were not pure, since there was considerable morning glory vines and other weeds present. The orchard grass was growing slowly and below average in green color due to drought conditions. Clippings were hand raked which introduced a variable amount of last year's crop, which had been badly weathered.

After a 7-day preliminary period a 5-day digestion trial was carried out. Digestibility results were reported according to the 4 different methods used: (1) total collection, (2) experimental or pheophytin, (3) plant pigment ratio, and (4) Reid's formula, $Y = 32.74 + 0.0168\ X - 8.47 \log X$ where Y = digestibility and X = units of plant pigment per gram of feces (dry weight).

Dry Matter Digestibility Coefficients

Cow	Total Collection	Pheophytin	Plant Pigment Ratio	Reid's Formula
286	56.3	56.8	59.4	57.6
805	58.6	57.2	60.9	57.8
813	61.4	60.8	61.6	58.2
Ave (3)	58.8	58.3	60.6	57.9

The above experiment was duplicated using a third cutting of a clover mixture. At the time of cutting the red clover was the predominant plant and was in the seed stage. The forage was harvested by a field chopper so that contamination with dead stubble was avoided.

Digestibilities obtained on the clover mixture on a 5-day trial were as follows:

Dry Matter Digestibility Coefficients

Cow	Total Collection	Pheophytin	Plant Pigment Ratio	Reid's Formula
297	64.6	64.1	71.2	61.3
298	61.8	63.6	70.0	61.0
813	64.5	65.9	73.0	61.9
Ave (3)	63.7	64.5	71.4	61.4

A third trial was conducted on alfalfa silage. In this experiment the chlorophyll in the alfalfa silage had been changed naturally to pheophytin or possibly lower degradation products of chlorophyll by the acid and heat developed by the silage fermentation. Addition of oxalic acid was found to be unnecessary in the case of silage and the only difference between Reid's plant pigment method and the pheophytin experimental method was that the latter samples were read at 415 millimicrons instead of 406 millimicrons.

Cow 900 went off feed one day during the trial and displayed below average feed consumption and diarrhea for one day. This apparently affected the digestibility values obtained with this animal and while her data is reported, it is not computed in reporting average values for total collection.

Digestibility values on an alfalfa silage ration for a three day period are:

Cow	Conventional	Pheophytin	Plant Pigment	Reid's Formula
836	56.0	57.6	44.6	43.1
900	47.6	59.1	46.0	42.9
906	56.6	59.8	45.3	43.0
Ave (2)	56.3	58.7	45.3	43.0

Conclusions:

For pasture forages the pheophytin method appears to be an excellent method of determining digestibility. For the six animals studied on orchard grass and mixed clover the average value for the conventional method was 61.2 while the experimental method (pheophytin) was 61.4.

For silage studies based on the results of 2 animals, the pheophytin method seems to yield values closer to the conventional than those obtained by Reid's chromogen method.

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